AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 2, line 20 with the following text:

Further, JP Kokai No.6-502548 discloses an expression system and a secretion system of Corynebacterium comprising a Corynebacterium strain and a secretory cassette comprising the first functional DNA sequence for the expression in the strain, the second DNA sequence encoding for amino kids, polypeptides and/or proteins and the third DNA sequence inserted between the first DNA sequence and the second DNA sequence, wherein the third DNA sequence encodes the protein element selected from PS1 and PS2 which guarantee the secretion of the amino acids, polypeptides and/or proteins. Specifically, the secretion of polypeptides is disclosed therein and in particular, NTG ~~mutagtenesis~~ mutagenesis was conducted with Corynebacterium and a mutant resistant to 4-fluoroglutamate (4FG) which is an analogue to glutamate is selected and subjected to the transformation with POGL141. It is described therein that a strain having an enhanced expression of GDH can be obtained from the analogue resistant bacteria. It is also described therein that a mutation was observed in nucleotide sequence No.251 to No.266 of GDH promoter.

Please replace the paragraph beginning on page 6, line 18 with the following text:

Effective ~~enzumes~~ enzymes for arginine fermentation include N-acetylglutamate synthase, N-acetylglutamate kinase, N-acetylglutamyl phosphate reductase, acetylornithine aminotransferase, N-acetylornithinase, ornithine carbamyltransferase, argininosuccinate synthase, and arginosuccinase. ~~arginine~~ Arginine is formed by the reaction catalyzed by these enzymes. These enzymes are effective. These enzymes are coded by enzymes argA, argB, argC, argD, ~~aegE~~ argE, argF, argG and argH, ~~relatively~~ respectively.

Please replace the paragraph beginning on page 9, line 10 with the following text:

It is particularly preferred in the present invention that the DNA sequence in -35 region of the ~~prompter~~ promoter for GDH-producing gene is at least one DNA sequence

selected from the group consisting of CGGTCA, TTGTCA, TTGACA and TTGCCA and/or the DNA sequence in -10 region of the promoter is TATAAT, or the bases of ATAAT in TATTAT sequence in -10 region is replaced with another base, while they do not inhibit the promoter function. The reason why the strain in which the bases of ATAAT in TATAAT sequence in -10 region is replaced with another base and the promoter function is not inhibited can be selected is as follows: Because a remarkable increase in the specific activity of GDH was observed by merely replacing the first "C" of CATAAT with "T" in wild type -10 sequence (refer to p6-4 in Table 1), it was considered that such a replacement with another base is possible.

Please replace the paragraph beginning on page 10, line 17 with the following text:

The promoters for algininosuccinate synthase are those having at least one DNA sequence selected from the group consisting of TTGCCA, TTGCCA, and TTGCCA in -35 region and/or TATAAT sequence in -10 region, or the base of ATAAT in TATTAT sequence is replaced with another base, which do not inhibit the function of the promoter. Argininosuccinate synthase ~~gene~~ genes having the above-described promoter are also provided.

Please replace the paragraph beginning on page 11, line 18 with the following text:

L-glutamic acid thus produced and accumulated in the culture liquid is collected by an ordinary method such as ion-exchange resin method or crystallization method. ~~Specifrrfically~~ Specifically, L-glutamic acid is separated by the adsorption on an anion-exchange resin or by the neutralization crystallization.

Please replace the paragraph beginning on page 12, line 20 with the following text:

As shown in Fig. 1, a chromosomal gene of a wild type strain of a coryneform bacterium ATCC13869 prepared with "Bacterial Genome DNA purification kit" (Advanced Genetic Technologies Corp.) was used as the template for PCR. The gene amplification was

conducted by PCR using upstream and downstream sequences of GDH gene. Both ends were blunt-ended. The product thus obtained was inserted in SmaI site of plasmid pHSG399 (a product of Takara Shuzo Co., Ltd.). Then a replication origin taken from plasmid pSAK4 having the replication origin capable of replicating in a coryneform bacterium was introduced into SalI site of the plasmid to obtain plasmid pGDH. By this method, GDH genes having each above-described promoter sequence can be obtained by using a primer having each of the sequence of Seq ID No. 1 to Seq ID No. 6 shown in the Sequence Listing as the upstream primer for GDH gene, respectively. It was confirmed by sequencing the PCR amplified fragment that any mutation, other than the introduced mutation in the promoter sequence, ~~was~~ did not ~~occured~~ occur in the amplified fragment. pSAK4 is constructed as follows: previously obtained plasmid pHK4 (J. P. KOKAI No.5-7491] having an autonomous replication origin derived from plasmid pHM1519 [Agric. Biol. Chem., 48, 2901-1903 (1984)] which is capable of autonomously replicating in Corynebacterium microorganism, is digested with restriction enzymes BamHI and KpnI to obtain a DNA fragment having the replication origin. Then the fragment thus obtained is blunt-ended with DNA-Blunting Kit (Blunting kit of Takara Shuzo Co., Ltd.). After the ligation with SalI linker, the product thus obtained was inserted into Sal I site of pHSG299 (a product of Takara Shuzo Co., Ltd.) to obtain plasmid pSAK4.

Please replace the paragraph beginning on page 15, line 1 with the following text:

AJ13029 were cultured on a CM2B agar medium (Table 2) at 31.5°C for 24 hours to obtain the bacterial cells. The cells were treated with 250μg/ml aqueous solution of N-methyl-N'-nitro-N-nitrosoguanidine at 30°C for 30 minutes. Then a suspension of the cells having a survival rate of 1 % was spread on agar plates culture medium (Table 3) containing 4-fluoroglutamic acid (4FG). Colonies were formed after incubating the plate at 31.5°C for

20 to 30 hours. In this experiment, a slant medium containing 1 mg/ml of 4FG was prepared at first, and then a layer of the same medium without 4FG was formed thereon horizontally. Thus, 4FG concentration gradient was obtained on the surface of the agar medium. When the plate was inoculated with the mutant cells obtained as described, a boundary line was formed at a border of the growing limit of the strain. Bacterial trains which formed colonies in ~~a area~~ an area containing 4FG of a concentration higher than that of the boundary line were taken. Thus, about 50 strains resistant to 4FG were obtained from about 10,000 mutagenized cells.

Please replace the paragraph beginning on page 18, line 9 with the following text:

The sequence of gltA gene of a coryneform bacterium, which codes citrate-synthesizing enzyme, has already been elucidated [Microbial. 140, 1817-1828 (1994)]. On the basis of this sequence, primers shown in Seq ID No. 7 and Seq ID No. 8 were synthesized. On the other hand, chromosomal DNA from Brevibacterium lactofermentum ATCC13869 was prepared using Bacterial Genome DNA Purification Kit (Advanced Genetic Technologies Corp.). Sterilized water was added to a mixture of 0.5 μg of the chromosomal DNA, 10 pmol of each of the oligonucleotides, 8μl of dNTP mixture (2.5 mM each), 5μl of 10xLa Taq Buffer (Takara Shuzo Co., Ltd.) and 2 U of La Taq (Takara Shuzo Co., Ltd.) to obtain 50μ1 of PCR reaction cocktail. The reaction cocktail was subjected to PCR. The PCR conditions were 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 15 seconds and ~~extention~~ extension at 72°C for 3 seconds using Thermal Cycler TP240 (Takara Shuzo Co., Ltd.) to amplify about 3 Kbp of DNA fragments containing gltA gene and promoter thereof. The amplified ~~fragements~~ fragments thus obtained were purified with SUPRECO2 (Takara Shuzo Co., Ltd.) and then blunt-ended. The blunting was conducted with Blunting Kit of Takara Shuzo Co., Ltd. The blunt-ended fragment was mixed with pHSG399 (Takara Shuzo Co., Ltd.) completely digested with SmaI to conduct the ligation.

The ligation reaction was conducted with DNA Ligation Kit ver 2 (Takara Shuzo Co., Ltd.). After the completion. of the ligation, the transformation was conducted with competent cells of E. coli JM109 (Takara Shuzo Co., Ltd.). The cells were spread on an L medium plates (comprising 10 g/l of bactotryptone, 5 g/l of bactoyeast extract, 5 g/l of NaCI and 15 g/l of agar; pH 7.2) containing 10 μg/ml of IPTG (isopropyl-β-D-thiogalactopyranoside), 40μg/ml of X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) and 40 μg/ml of chloramphenicol. After culturing them overnight, white colonies were taken to obtain the transformed strains after single colony isolation.

B9 Cont

Please replace the paragraph beginning on page 19, line 12 with the following text:

Mutan-Super Express Km (Takara Shuzo Co., Ltd.) was used for the introduction of mutation into gltA promoter region. The method is ~~speciffically~~ specifically described below. PHSG399CS was completely ~~digestied~~ digested with EcoRI and SalI to obtain EcoRI-SalI fragment containing gltA genes, which were ligated to the fragment obtained by complete digestion of pKF19kM (Takara Shuzo Co., Ltd.) with EcoRI and SalI. After the completion of the ligation, the transformation was conducted with competent cells of E. coli JM109 (Takara Shuzo Co., Ltd.). The cells ~~was~~ were spread on L medium plates containing 10 μg/ml of IPTG, 40μg/ml of X-Gal and 25 μg/ml of kanamycin. After overnight incubation, white colonies were taken and transformants were obtained by single colony isolation. From the transformants, plasmids were prepared and the plasmid containing gltA gene was named pKF19CS.

B10

Please replace the paragraph beginning on page 20, line 18 with the following text:

pKF19CS, pKF19CS1, pKF19CS2 and pKF19CS4 constructed in step (2) were completely digested with SalI and EcoRI (Takara Shuzo Co., Ltd.). On the other hand, plasmid pSFK6 (Japanese Patent Application No.11-69896) having a replication origin

B11

derived from plasmid pAM330 which can autonomously replicate in a coryneform bacterium [Japanese Patent Publication for Opposition Purpose (hereinafter referred to as "J. P. KOKAI") No. 58-67699] was completely digested with EcoRI and SalI. The obtained fragment was ligated with about the 2.5 kb fragment containing gltA. After the completion of the ligation, transformation was conducted with competent cells of E. coli JM109. The cells ~~was~~ were spread on the L-medium plates containing 10 μg/ml of IPTG, 40 g/ml of X-Gal and 25 μg/ml of kanamycin. After overnight incubation, colonies were taken and the transformants were obtained after single colony isolation. From the transformants, plasmids were prepared. The plasmids containing gltA gene were named pSFKC, pSFK1, pSFKC2 and pSFKC4, respectively.

Please replace the paragraph beginning on page 21, line 5 with the following text:

The plasmid constructed in above step (3) was introduced into Brevibacterium lactofermentum ATCC13869. Specifically, this treatment was conducted by electrical pulse method (J. P. KOKAI No. 2-07791). The transformants were selected at 31°C with CM2B medium plate (comprising 10 g/1 of bactotryptone, 10 g/1 of bactoyeast extract, 5 g/1 of NaCl, 10μg/I of biotin and 15 g/1 of agar; pH 7.0) containing 25 μg/ml of kanamycin. After incubating for two days, colonies were taken and the transformants containing pSFKC, pSFK1, pSFKC2 and pSFKC4 were named BLCS, BLCS1, BLCS2 and BLCS4, respectively, after single colony isolation. A medium having a composition shown in Table 8 was inoculated with the transformant. The culture was continued at 31°C and terminated before the glucose had been completely consumed. The culture liquid was centrifuged to separate the cells. The cells were washed with 50 mM tris buffer solution (pH 7.5) containing ~~200m~~ 200mM of sodium glutamate and then suspended in the same buffer solution. After the sonication with UD-201 (TOMY) followed by the centrifugation

(10,000g), the cells remaining unbroken were removed to obtain a crude enzyme solution. The activity of citrate synthase can be determined according to Methods Enzymol. 13, 3-11 (1969). Specifically, the crude enzyme solution was added to a reaction mixture containing 100 mM of ~~TisHCl.~~ TrisHCl (pH 8), 0.1 mM~~,~~ of DTNB, 200 mM of sodium glutamate and 0.3 mM of acetyl CoA, and the background was determined as the increase in the absorbance at 412 nm at 30°C determined by Hitachi spectrophotometer U-3210. Further, oxaloacetic acid was added in such an amount that the final concentration thereof would be 0.5 mM. The increase in the absorbance at 412 nm was determined, from which the background value was deducted to determine the activity of the citrate synthase. The protein concentration in the crude enzyme solution was determined by Protein Assay (BIO-RAD.). Bovine serum albumin was used as the standard protein. The results are shown in Table 9. It was confirmed that the citrate synthase activity of mutant gltA promoters was increased compared to wild-type gltA.

B12 Cont

Please replace the paragraph beginning on page 24, line 24 with the following text:

The activities of the citrate synthase were determined by treating FGR2, GB01, GB02, GB03 and FGR2/pSFKC strains obtained in step (7) in the same manner as that of step (4). The results are shown in Table 10. It was confirmed that the citrate synthase activity of the ~~substitited~~ substituted gltA promoter strain was higher than that of the parent strains thereof.

B13

Please replace the paragraph beginning on page 25, line 22 with the following text:

As a result, the larger improvement in the yield of L-glutamic acid was confirmed when each of the strains GB02 and GB03 rather than GB01 and FGR2/pSFKC was used as shown in Table 12. From these facts, it was found that good results were obtained by introducing the mutation into the ~~gttA~~ gltA promoter to increase the CS activity to 2 to 4 times for the improvement in the yield of glutamic acid produced by those strains.

B14

Please replace the paragraph beginning on page 28, line 5 with the following text:

PCR was conducted by using pKF18icd as the template and 5'-phosphorylated synthetic DNA shown in Seq ID No. 16, Seq ID No. 17, Seq ID No. 18, Seq ID No. 19, Seq ID No. 20 and Seq ID No. 21 and the selection primer. These PCR products were used for transforming competent cells of E. coli JM109. The cells were spread on the L-medium plates containing 25 μg/ml of kanamycin. After overnight incubation, formed colonies were taken and the transformants were obtained after single colony isolation. From the transformants, plasmid DNA was prepared, and the sequence of icd promoter region was determined using synthetic DNA shown in Seq ID No. 22 by Sanger's method [J. Mol. Biol., 143, 161 (1980)]. Specifically, the DNA sequence was determined with Dye Terminator Sequencing Kit (Applied Biosystems), and analyzed with Genetic Analyzer AB1310 (Applied Biosystems). Those obtained by replacing icd promoter region with a sequence shown in Table 7 were named pKF18ICD1, pKF18ICD2, pKF18ICD3, pKF18ICD4, pKF18ICD5 and pKF18ICD6m respectively. Among them, pKF18ICD2 was completely digested with PstI to obtain PstI fragment containing the promoter of icd gene. The fragment was ligated with the fragment obtained by complete PstI digestion of pKF18kM (Takara Shuzo Co., Ltd.). After the completion of the ligation, the transformation was conducted with competent cells of E. coli JM109 (Takara Shuzo Co., Ltd.). The cells were spread on the L-medium plates containing 10 μg/ml of IPTG, 40 μg/ml of X-Gal and 25 μg/ml of kanamycin. After overnight ~~incubaton~~ incubation, white colonies were taken and the transformed strains were ~~obatined~~ obtained after single colony isolation. From the transformed strains, plasmids were prepared, and the plasmid containing the promoter of icd gene was named pKF18ICDM2. PCR was conducted using pKF18ICDM2 as the template and ~~5' phosphorilated~~ 5'-phosphorylated synthetic DNA shown in Seq ID No. 20 and Seq ID No.21 and the selection primer. The transformation of competent cells of E. coli JM109 was

conducted with the PCR product. The cells were spread on the L-medium plates containing 25 μg/ml of kanamycin. After overnight incubation, colonies thus formed were taken and transformants were ~~obatined~~ obtained after single colony isolation. From the transformants, plasmids DNA were prepared, and the sequence of icd promoter region was determined using synthetic DNA shown in Seq ID No. 22. Those obtained by replacing icd promoter region with the sequence shown in Table 13 were named pKF18ICD25 and pKF18ICD26, ~~repectively~~ respectively.

Please replace the paragraph beginning on page 30, line 29 with the following text:

Plasmids having mutant icd promoter constructed in above-described step (2), i.e. pKF18ICD1, pKF18ICD2, pKF18ICD3, pKF18ICD4, pKF18ICD5, pKF18ICD6, pKF18ICD25, pKF18ICD26 and pKF18ICD, were completely ~~digestede~~ digested with SacII and PstI and then blunt-ended. They were ligated with fragment obtained by digesting pNEOL with SmaI. After the completion of the ligation, the transformation was conducted with competent cells of E. coli JM109. The cells were spread on the L-medium plates containing IPTG, X-Gal and 40 μg/ml of chloramphenicol. After overnight incubation, blue colonies were taken and the transformed strains were obtained after single colony isolation.

Please replace the paragraph beginning on page 32, line 22 with the following text:

Plasmid vector pSFKT2 (Japanese Patent Application No. 11-81693) the replication of which in a coryneform bacterium was temperature-sensitive was used. pKF18ICD1, pKF18ICD2, pKF18ICD3, pKF18ICD4, p,KF18ICD5, pKF18ICD6, pKFICD25 and pKFICD26 were completely digested with PstI and the obtained fragments were used as the mutant icd promoter sequences. The fragments thus obtained were ligated with pSFKT2 completely digested with PstI. After the completion of the ligation, the transformation was conducted with competent cells of E. coli JM109 (Takara Shuzo Co., Ltd.). The cells were spread on the L-medium plates containing 10 μg/ml of IPTG, 40 μg/ml of X-Gal and 25

μg/ml of kanamycin. After overnight incubation, white colonies were taken and transformed strains were obtained after single colony ~~isloation~~ isolation. From the transformed strains, plasmids were prepared. Temperature-sensitive shuttle vectors containing icd promoter were named pSFKTI1, pSFKTI2, pSFKTI3, pSFKTI4, pSFKTI5, pSFKTI6, pSFKTI25 and pSFKTI26, respectively.

Please replace the paragraph beginning on page 33, line 7 with the following text:

(6) ~~Integreation~~ Integration of mutant icd promoter into chromosome:

Please replace the paragraph beginning on page 34, line 20 with the following text:

ICDH crude enzyme solution was prepared by using each of the 8 strains obtained in above-described step (7) and GB02 strain in the same manner as that of step (7) in Example 3. The ICDH activities were determined as follows: The crude enzyme solution was added to a reaction solution containing 35 mM of ~~TisHCl~~ Tris-HCl (pH 7.5), 1.5 mM of $MnSO_4$, 0.1 mM of NADP and 1.3 mM of isocitric acid, and the increase in the absorbance at 340 nm at 30°C was determined with Hitachi spectrophotometer U-3210 as the activity of ICDH. The protein concentration in the crude enzyme solution was determined by Protein Assay (BIO-RAD). Bovine serum albumin was used as the standard protein. The results are shown in Table 15. It was confirmed that the isocitrate dehydrogenase activity of substituted icd promoter strains was higher than that of the parent strain.

Please replace the paragraph beginning on page 36, line 10 with the following text:

Primers shown in Seq ID No.25 and Seq ID No.26 were synthesized by selecting regions having a high homology among EI subunits of pyruvate dehydrogenase (PDH) of Escherichia coli, Pseudomonas aeruginosa and Mycobacterium tuberculosis. PCR was conducted by using chromosome of Brevibacterium lactofermentum ATCC13869, prepared with a bacterial genomic DNA purification kit (Advanced Genetic Technologies Corp.), as the template under standard reaction conditions described on page 8 of PCR Technology

(edited by H. Erlich and published by Stockton Press, 1989). The reaction solution was subjected to the electrophoresis in an agarose gel to find that about 1.3 kilobases of DNA fragment was amplified. The sequence of both end of the obtained DNA was determined with synthetic DNA shown in Seq ID No. 25 and Seq ID No.26. The sequence was determined by Sanger's method [J. Mol. Biol., 143, 161 (1980)] with DNA Sequencing Kit (Applied Biosystems Co.). The determined sequence was deduced to amino acids, and compared with E1 subunits of pyruvate dehydrogenase derived from each of Escherichia coli, Pseudomonas aeruginosa and Mycobacterium tuberculosis to find a high homology among them. Consequently, it was ~~dtermined~~ determined that the DNA fragment amplified by PCR was a part of pdhA gene which codes EI subunit of pyruvate dehydrogenase of Brevibacterium lactofermentum ATCC13869. The cloning of the upstream and downstream region of the gene was conducted. The cloning method was as follows: A chromosome of Brevibacterium lactofermentum ATCC13869 was digested with restriction enzymes EcoRI, BamHI, Hind III, Pst I, Sal I and Xba I (Takara Shuzo Co., ltd.) to obtain DNA fragments. LA PCR in vitro cloning Kit (Takara Shuzo Co., Ltd.) was used for the cloning, using the sequences shown in Seq ID No. 27 and Seq ID No. 28 in the Sequence Listing as primers for cloning the upstream region, and sequences shown in Seq ID No. 29 and Seq ID No. 30 as primers for cloning the downstream region. After PCR using the kit, DNA fragments of about 0.5, 2.5, 3.0, 1.5 and 1.8 kilobases were amplified for the upstream region from the fragments obtained by digestion with EcoRI, Hind III, Pst I, Sal I and Xba I, ~~respectrively~~ respectively; and DNA fragments of about 1.5, 3.5 and 1.0 kilobase were amplified for the downstream region from the ~~fragements~~ fragments obtained by digestion with BamHI, Hind III and Pst I, respectively. The sequences of these DNA fragments were determined in the same manner as that described above. It was found that the amplified DNA fragments further contained an open reading frame of about 920 amino acids and also that a region supposed to

be a promoter region was present in the upstream region. Because the deduced amino acid sequence from the DNA sequence of the open reading frame is highly homologous to known EI subunit of pyruvate dehydrogenase such as that of E. coli, it was apparent that the open reading frame was the pdhA gene which codes EI subunit of pyruvate dehydrogenase of Brevibacterium lactofermentum ATCC13869. The DNA sequence of the open reading frame was shown in Seq ID No. 31 in the Sequence Listing. In Seq ID No. 31 in the Sequence Listing, deduced amino acid sequence from the DNA sequence is also shown. Since methionine residue at N-terminal of the protein is derived from ATG which is an initiation codon, it usually does not concern the essential function of protein, and it is well known that the methionine residue is removed by the effect of peptidase after the translation. Therefore, in the above-described protein, it is possible that methionine residue at the N-terminal has been removed. However, the GTG sequence is present in 6 bases upstream of ATG shown in Seq ID No. 31 in the Sequence Listing, and it is also possible that amino acids is translated from this point. Pyruvate ~~dehydrogenase~~ dehydrogenases of other microorganisms such as E. coli are composed of three subunits of E1, E2 and E3, and genes which encode them constitute an operon in many cases. However, there was no open reading frame considered to be E2 and E3 subunit of pyruvate dehydrogenase in about 3 kilobases downstream of pdhA gene. Instead, it was shown that a sequence supposed to be a terminator was present in the downstream of the open reading frame. From these facts, it was supposed that E2 and E3 subunits of pyruvate dehydrogenase of Brevibacterium lactofermentum ATCC13869 were present in another ~~regon~~ region on the chromosome.

Please replace the paragraph beginning on page 39, line 11 with the following text:

From the ~~transformned~~ transformed strains, plasmids were prepared by alkali method (*Seibutsu Kogaku Jikken-sho* edited by Nippon *Seibutsu Kogaku kai* and published by *Baifukan*, p. 105, 1992). Restriction enzyme maps of DNA fragments inserted into the

vectors were prepared and compared with the restriction enzyme map of pdhA gene reported in sequence No. 32 of the Sequence Listing. A plasmid containing DNA fragments inserted therein having the same restriction enzyme map as that of pdhA gene was named pSFKBPDHA.

Please replace the paragraph beginning on page 39, line 21 with the following text:

Brevibacterium lactofermentum ATCC13869 and GC25 were transformed with plasmid pSFKBPDHA by electrical pulse method (J. P. KOKAI No.2-207791) to obtain the transformed strains. The culture for producing L-glutamic acid was conducted with transformed strain ATCC13869/pSFKBPDHA and GC25/pSFKBPDHA obtained by introducing plasmid pSFKBPDHA into Brevibacterium lactofermentum ATCC13869 and GC25 as follows: Cells of ATCC13869/pSFKBPDHA and GC25/pSFKBPDHA obtained by the culture on CM2B medium plates containing 25 μg/ml of kanamycin were inoculated into a medium (comprising 1 liter of pure water containing 80 g of glucose, 1 g of KH_2PO_4, 0.4 g of $MgSO_4$ $7H_2O$, 30 g of $(NH_4)_2SO_4$, 0.01 g of $FeSO_4$ $7H_2O$, 0.01 g of $MnSO_4$ $7H_2O$, 15 ml of soybean protein hydrolyzate, 200 μg of thiamine hydrochloride, 60 μg of biotin, 25 mg of kanamycin and 50 g of $CaCO_3$; and having a pH adjusted to 8.0 with KOH). Then the culture was ~~shaked~~ shaken at 31.5°C until sugar in the medium had been consumed. The obtained products were inoculated into the medium of the same composition as that described above (for GC25/pSFK6 and GC25/pSFKBDHA) or the medium eliminated Biotin from the composition as that described ~~above(for~~ above (for ATCC13869/pSFK6 and ATCC13869/pSFKBPDHA) in an amount of 5%, and the shaking culture was conducted at 37°C until sugar in the medium had been consumed. As a control, strains obtained by transforming Brevibacterium lactofermentum ATCC13869 and GC25 with previously obtained ~~palsmid~~ plasmid pSFK6 capable of autonomously replicating in coryneform bacterium by electrical pulse method (J. P. KOKAI No. 2-207791), were cultured in the

same manner as that described above. After the completion of the culture, the amount of L-glutamic acid accumulated in the culture medium was determined with Biotic Analyzer AS-210 (a product of Asahi Chemical Industry Co., Ltd.). The results are shown in Table 17.

Please replace the paragraph beginning on page 42, line 14 with the following text:

Further, primers indicated as Seq ID No. 37, Seq ID No. 38 and Seq ID NO. 39 in the Sequence Listing were synthesized for constructing plasmids wherein a region supposed to be the promoter site was changed to the consensus sequence of promoters of coryneform bacteria. By using each of the primers and a primer shonw in Seq ID No. 36, DNA fragments wherein the promoter region of pdhA gene was changed to the consensus sequence were amplified by PCR method by using chromosomal DNA of Brevibacterium lactofermentum ATCC13869 as a template. Among the synthesized primers, Seq ID No. 37 corresponded to the sequence ranging from base No. 2244 to base No. 2273 in Seq ID No. 32; base No. 2255 had been replaced with C, and base No. 2257 had been replaced with A; thus only -35 region had been changed to the consensus sequence of the coryneform bacteria. Seq ID No. 38 corresponded to the sequence ranging from base No. 2249 to base No. 2288 in sequence No. 32; base No. 2279 and No. 2281 had been replaced with T; thus only -10 region had been changed to the consensus sequence of the coryneform bacteria. Sequence No. 39 corresponded to the sequence ranging from base No. 2249 to base No. 2288 in Seq ID No. 32; base No. 2255 had been replaced with C, base No. 2257 had been replaced with A, and base No. 2279 and No. 2281 had been replaced with T; thus both -35 region and -10 region had been changed to the consensus sequence of the coryneform bacteria. PCR was conducted by using chromosome of Brevibacterium lactofermentum ATCC13869, prepared with a Bacterial Genomic DNA Purification Kit (Advanced Genetic Technologies Corp.), as the template under standard reaction conditions described on page 8 of PCR Technology (edited by H. Erlich and published by Stockton Press, 1989) to amplify the promoter region of pdhA

gene with these primers so that the promoter region was changed to the consensus sequence. PCR products thus obtained were purified by an ordinary method and reacted with restriction enzyme SmaI. The fragments were ligated with pNEOL lacking the promoter region of lacZ gene, which could replicate in a coryneform bacterium and which had been ~~cleavaged~~ cleaved with restriction enzymes Sma I, with a Ligation Kit (Takara Shuzo Co., Ltd.). After the transformation with competent cells (Takara Shuzo Co., Ltd.) of E. coli JM109, the cells were spread on the L-medium plates (comprising 10 g/l of bactotryptone, 5 g/l of bactoyeast extract, 5 g/l of NaCl and 15 g/l of agar and having pH 7.2) containing 40 μg/ml of X-Gal (5-bromo-4-chloro-3-indolyl- β-D-galactoside) and 25 μg/ml of kanamycin. After overnight incubation, blue colonies were taken and the transformed strains were ~~obatined~~ obtained after single colony isolation. From the transformed strains, plasmids were prepared by the alkali method (*Seibutsu Kogaku Jikken-sho* edited by *Nippon Seibutsu Kogaku kai* and published by *Baifukan*, p. 105, 1992). After sequencing DNA fragments inserted into the vector by an ordinary method, the plasmid containing DNA fragments, wherein only the sequence in -35 region had been changed to the consensus sequence, inserted therein was named pNEOLBPDHApro35; the plasmid containing DNA fragments, wherein only the sequence in -10 region had been changed to the consensus sequence, was inserted therein was named pNEOLBPDHApro10; and the plasmid containing DNA fragments, wherein the sequences in both -35 region and -10 region had been changed to the consensus sequence, was inserted therein was named pNEOLBPDHApro3510.

Please replace the paragraph beginning on page 44, line 1 with the following text:

(5) The ~~elavuation~~ evaluation of the mutated pdhA promoter activity:

Please replace the paragraph beginning on page 46, line 22 with the following text:

First, GC25 was transformed with plasmid pSFKTPDHApro3510 for preparing promoter modified strain by electrical pulse method (refer to J. P. KOKAI No. 2-207791).

The cells were spread on CM2B ~~mediu~~ media plates (comprising 10 g/l of polypeptone, 10 g/l of bactoyeast extract, 5 g/l of NaCl, 10 μg/ml of biotin and 15 g/l of agar, and having pH 7.2) and cultured at 25°C to obtain transformed strains. These transformants were cultured in CM2B liquid medium in a test tube overnight and then spread on CM2B medium plates containing 25 μg/ml of kanamycin and cultured at 34°C to obtain ~~a a strain~~ a strain caused by once-recombination which contains plasmid pSFKTPDHpro3510 on its chromosome inserted by the homologous recombination. After single colony isolation, this strain was cultured in CM2B liquid medium in a test tube overnight. After the suitable dilution, it was spread on CM2B medium plates and cultured at 31.5°C. After the colonies began to appear, the ~~repllicas~~ replicas were made on CM2B medium plates containing 25 μg/ml of kanamycin to obtain kanamycin-sensitive strains. Since two kinds of the strains, i.e. a strain having the sequence of wild type strain for the promoter region of pdhA gene and another strain having the mutation introduced therein, could be ~~occured~~ occurred, this region was sequenced. Thus, a promoter modified strain, wherein the mutation had been introduced into the promoter region of pdhA gene, was obtained. In this strain, -35 region and -10 region of promoter of pdhA gene had been changed to the consensus sequence of coryneform bacteria. This strain was named GD3510.

B23 Cont

Please replace the paragraph beginning on page 48, line 23 with the following text:

In order to amplify argG gene of Brevibacterium flavum by PCR, the DNA sequences in the upstream and downstream regions of the ORF were determined. The determination of the DNA sequences was conducted by synthesizing a primer based on the known DNA sequence (Gen Bank accession AF030520) of ORF of argG gene of Corynebacterium ~~glutamicom~~ glutamicum and using in vitro LA PCR cloning kit (Takara shuzo Co., Ltd.) in accordance with the instruction manual included in the kit. As primers, they were specifically used ~~oligonucleotide~~ oligonucleotides (primers 1 and 2) having the DNA

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sequences set out as Seq ID No. 42 and Seq ID No.43 for the upstream region, and ~~oligonucleotide~~ oligonucleotides (primers 3 and 4) having the DNA sequences set out as Seq ID No.44 and Seq ID No.45 for the downstream region. The DNA sequences in the upstream and downstream region of argG were determined by completely digesting chromosome DNA of 2247 strain (ATCC14067), i.e., wild type strain of Brevibacterium flavum, with a restriction enzyme EcoRl, conducting first PCR with the primer 2 or 3 (having sequence No. 43 or 44), and conducting second PCR with the primer 1 or 2 (having sequence No. 42 or 45).

Please replace the paragraph beginning on page 50, line 2 with the following text:

pNEOL-0, pNEOL-1, pNEOL-2, pNEOL-3, pNEOL-4 and pNEOL-7 were introduced into AJ12092 ~~strain,respectivly~~ strain, respectively. The plasmids were introduced by electrical pulse method (J. P. KOKAI No. 2-207791). The transformants were selected on CM2G medium ~~plates(comprising~~ plates (comprising 1 liter of pure water containing 10 g of polypeptone, 10 g of yeast extract, 5 g of glucose, 5 g of NaCl and 15 g of agar, and having pH 7.2) containing 4 μg/ml of chloramphenicol, as chloramphenicol-resistant strains.

Please replace the paragraph beginning on page 52, line 2 with the following text:

Based on the DNA sequence determined as in (1), oligonucleotides (primers 5 and 6) having the DNA sequence set out in Seq ID No. 46 and Seq ID No.47 were synthesized to conduct PCR using chromosomal DNA of Brevibacterium flavum as a template. The PCR reaction was conducted in 25 cycles, each cycle consisting of 94°C for 30 seconds, 55°C for one second and 72°C for 2 minutes and 30 seconds. The thus-obtained DNA fragment was cloned to SmaI site in multi-cloning site of cloning vector pSTV29 (Takara shuzo Co. Ltd.) ~~toobtain~~ to obtain pSTVargG. Furthermore, pargG was prepared by inserting into SalI site of pSTVargG a fragment containing the replication origin obtained by treating pSAK4 set out in Example 1 with SalI.

Please replace the paragraph beginning on page 52, line 13 with the following text:

pargG was introduced into the strain Brevibacterium ~~lactofernentum~~ lactofermentum AJ12092. Plasmid was introduced by electrical pulse method (J. P, KOKAI No. 2-207791). The transformant was selected as chloramphenicol-resistant strain on CM2G medium ~~plates(comprising~~ plates (comprising 1 liter of pure water containing 10 g of polypeptone, 10 g of yeast extract, 5 g of glucose, 5 g of NaCl and 15 g of agar, and having pH 7.2) containing 4 μg/ml of chloramphenicol.

Please replace the paragraph beginning on page 54, line 21 with the following text:

Plasmids having GDH promoter sequence of FGR1 strain and FGR2 strain described in Example 2 were constructed by site directed mutagenesis. For obtaining GDH promoter sequence of FGR1 strain, PCR was conducted by using synthetic DNA shown in Seq ID No. 57 and synthetic DNA shown in No. 60 as the primers and chromosomal DNA of ATCC13869 as the template; and on the other hand, PCR was conducted by using synthetic DNA shown in Seq ID No. 58 and synthetic DNA shown in Seq ID No. 59 as the primers with chromosomal DNA of ATCC13869 as the template. Further, PCR was conducted by using synthetic DNAs shown in Seq ID Nos. 57 and Seq ID No.58 as the primers with a mixture of these PCR products as the template. The PCR product thus obtained was inserted into SmaI site of pSFKT2 (Japanese Patent Application No. 11-69896) to construct pSFKTG11. To obtain GDH promoter sequence of FGR2 strain, PCR was conducted by using synthetic DNA shown in Seq ID No. 57 and synthetic DNA shown in Seq ID No. 62 as the primers and chromosomal DNA of ATCC13869 as the template; and on the other hand, PCR was conducted by using synthetic DNA shown in Seq ID No. 58 and synthetic DNA shown in Seq ID No. 61 as the primers and chromosomal DNA of ATCC13869 as the template. Further, PCR was conducted by using synthetic DNA shown in Seq ID No. 57 and Seq ID No. 58 as the primers and a mixture of these PCR products as the template. The PCR

product thus obtained was inserted into SmaI site of pSFKT2 (Japanese Patent Application No. 11-69896) to construct pSFKTG07. The DNA sequences of the fragments inserted into SmaI sites of pSFKTG11 and pSFKTG07 were determined to confirm that no mutation was introduced into other ~~refions~~ regions than the promoter region in GDH.

Please replace the paragraph beginning on page 57, line 10 with the following text:

pGDH and pAJ220G were introduced into AJ13029 by electrical pulse method. Each of these strains and those obtained in above-described step (2) was inoculated into a seed culture medium having a composition shown in Table 25, and the shaking culture was conducted at 31.5°C for 24 hours to obtain the seed culture. 300 ml of medium for main culture having a composition shown in Table 25 was placed in each of 500 ml glass jar ~~farmenters~~ fermenter and then sterilized by heating. 40 ml of the seed cultures as described above were inoculated into the medium. The culture was started at a temperature of 31.5°C while the stirring rate and the aeration rate were controlled at 800 to 1300 rpm and 1/2 to 1/1 vvm, respectively. The pH of the culture liquid was kept at 7.5 with gaseous ammonia. The temperature was shifted to 37°C 8 hours after the initiation of the culture. The culture was terminated when glucose had been completely consumed in 20 to 40 hours, and the quantity of L-glutamic acid produced and accumulated in the culture liquid were determined (Table 26). The GDH activity for obtaining the highest yield was about 3-times as high. When GDH activity was further elevated, the degree of the improvement in the yield was reduced. When the GDH activity was elevated to about 16-times, the yield was rather reduced. Amino acids produced as by-products were analyzed with Hitachi Amino Acid Analyzer L-8500 to find that as GDH activity was elevated, the amount of accumulated aspartic acid and alanine was increased. These results proved the following facts: For increasing the yield of glutamic acid, it is necessary to suitably increase GDH activity so as not to cause a remarkable increase in the amount of aspartic acid and alanine. One of the effective methods therefor

comprises the introduction of various mutations into gdh promoter to control GDH activity to about 3-times as high as that of the parent strain.